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Cystic structures in fish ovaries: more common than we think. The case study of *Sarpa salpa* (Sparidae)

by

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Résumé. – Structures kystiques dans les ovaires de poissons : une fréquence plus importante qu'imaginée. Le cas de *Sarpa salpa*.

Les ovaires de la saupe, *Sarpa salpa* (Linnaeus, 1758), présentent des structures dures et facilement visibles macroscopiquement. Leur analyse histologique montre que ce sont des masses d'ovocytes hydratés résultant d'un événement de ponte, incomplète ou non. Le modèle linéaire généralisé (MLG) montre que la présence de kystes a été significativement affectée selon les mois. Le fait que ces structures apparaissent fréquemment avec une prévalence élevée peut suggérer la présence d'un lien, ou d'une association, avec la stratégie de reproduction chez cette espèce.

Key words. – Sparidae - *Sarpa salpa* - Portugal - Cystic structures - Atresia - Hydrated oocytes.

The morphological characteristics of follicular atresia in fish ovary are known for more than three decades (Babin *et al*., 2007), but the cellular and molecular aspects of follicular atresia involving the degenerative processes of the oocyte and the follicular wall is not completely understood (Miranda *et al*., 1999). The presence of atretic follicles in the teleost ovary can be associated with stress, fasting, biocidal agents, light, temperature, confinement and inadequate hormone levels (Miranda *et al*., 1999). Atresia is present in all species and may affect all types of oocytes. Its intensity is usually low throughout the year, although it may increase during spawning season, especially in species with indeterminate fecundity that usually present mass atresia events at the end of that period (Murua and Saborido-Rey, 2003). Changes in the atretic process caused by an inflammation of the follicular envelope, leading to the formation of cysts at different stages of follicular development have been reported recently (Domínguez-Petit *et al*., 2011), although atresia of hydrated oocytes is considered rare and is poorly documented (Rideout and Burton, 2000; Brown-Peterson *et al*., 2007; Alonso-Fernández, 2011; Madureira *et al*., 2011).

This study focuses on an eurytherm sparid fish, salema *Sarpa salpa* (Linnaeus, 1758), a protandrous hermaphrodite (Criscoli *et al*., 2006). Information on reproduction of *S. salpa* is available only for the Canary Islands and is based only on macroscopic analysis of the gonads (Villamil *et al*., 2002). The present work is the first study describing the morphology of cystic structures in *S. salpa* ovaries and analysing the prevalence of aggregated cystic structures throughout the year and its relation to specimen size and sexual maturity stage.

MATERIALS AND METHODS

Sampled individuals were obtained monthly between January 2012 and July 2013 from artisanal fisheries in the central region of the Portuguese coast.

Because this species is a protandric hermaphrodite, only individuals larger than 28 cm of total length (TL) (females) were considered. TL (± 1 mm) and gut weight (WG ± 0.01 g) were registered. Ovaries were removed, weighed (WO ± 0.01 g) and stored in a 10% formaldehyde solution buffered at pH = 7 with carbonates (CH_2O; $M = 30.03$; $D = 1.02\ g/cm^3$). Tissue samples were dehydrated in a graded ethanol series (70-96%) and embedded either in methacrylate resin or in paraffin, following standard procedures. Methacrylate resin and paraffin sections of 3 μm were stained with toluidine blue and haematoxylin and eosin, respectively. The sexual maturity stage of each individual was histologically assigned according to Brown-Peterson *et al*. (2011). The gonadosomatic index (IG) was calculated according to the following expression: $IG = [(WO)\ (WG)^{-1}]*100$. Also Fulton condition factor (K) was estimated as $K = (WG)\ (TL^3)^{-1}$.

The cyst prevalence, defined as the percentage of females with aggregated cystic structure in the ovary relatively to the total number of sampled mature females, was estimated by month and TL of females. Analyses of variance using the generalised linear model (GLM) were conducted to test the influence of month, IG, K and TL on cyst presence (the analyses were performed using the software Statistica 11).

RESULTS

A total of 239 females were sampled, ranging from 28.6 to 44.6 cm TL (Tab. I). Histological observations showed that all females were sexually mature. Macroscopically, the ovaries

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Table I. - Mean ± standard deviation (S.D.) of the total length (TL) and gonadosomatic index (IG), cyst prevalence and number of *Sarpa salpa* females (*n*).

Month	Mean TL (cm) ± S.D.	Mean IG ± S.D.	Cyst prevalence (%)	*n*
January	35.69 ± 3.64	0.55 ± 0.10	3.30	33
February	36.20 ± 2.78	0.68 ± 0.20	26.09	23
March	35.43 ± 1.86	0.71 ± 0.20	43.75	16
April	36.29 ± 2.10	0.56 ± 0.10	41.67	12
May	36.09 ± 1.90	0.62 ± 0.10	44.44	18
June	33.70 ± 1.84	0.62 ± 0.10	50.00	2
July	36.79 ± 2.58	0.66 ± 0.20	66.67	15
August	36.88 ± 1.97	1.18 ± 0.50	19.05	21
September	37.11 ± 2.15	2.68 ± 0.50	5.26	19
October	36.61 ± 1.81	1.15 ± 0.60	0.00	17
November	36.42 ± 1.80	0.79 ± 0.50	17.95	39
December	38.10 ± 2.65	0.71 ± 0.20	33.33	24
			Total	239

revealed the presence of hard structures with a yellow to brown colour (Fig. 1A) in 24.3% of the females sampled. These structures could correspond to remains of hydrated oocytes that can appear either isolated or in groups joining together, forming a cystic structure (Fig. 1B, C) corresponding to an incomplete or unsuccessful spawning event. Cysts seemed to be formed by numerous zona radiata membranes folded over one another and surrounded by a stratified epithelium (Fig. 1D). Macrophages were also observed in the cyst envelope (Fig. 1C).

The cysts were found all months (Tab. I), except in October, and the mean prevalence value was higher (ranging between 40 and 67%) in months preceding the spawning period, i.e. August to November (R.B. Paiva, pers. obs.). The greater prevalence of these structures (45.2%) was found in the 37-38 cm TL class. Regarding the maturity stages, the cysts were only observed in the regenerating stage (according to the classification of Brown-Peterson *et al.*, 2011), affecting 36.0% of females in this stage. The GLM showed that the presence of cysts was significantly affected by month (GLM: $F = 2.921$; d.f. = 11; $p < 0.05$) and was affected neither by TL (GLM: $F = 0.007$; d.f. = 1; $p = 0.93$), nor IG (GLM: $F = 0.013$; d.f. = 1; $p = 0.90$) nor K (GLM: $F = 0.307$; d.f. = 1; $p = 0.58$).

DISCUSSION

In teleost fish, the presence of remains of hydrated oocytes, isolated or aggregated in the ovary, has been reported sporadically, e.g. in zebrafish *Danio rerio* (Hamilton, 1822) (Madureira *et al.*, 2011), Atlantic cod *Gadus morhua* Linnaeus, 1758 (Rideout and Burton, 2000), Atlantic blue marlin *Makaira nigricans* Lacepède, 1802 (Brown-Peterson *et al.*, 2007) and pouting *Trisopterus luscus* (Linnaeus, 1758) (Alonso-Fernández, 2011), and has been related to abnormal atresia due to changes in the energy requirement of

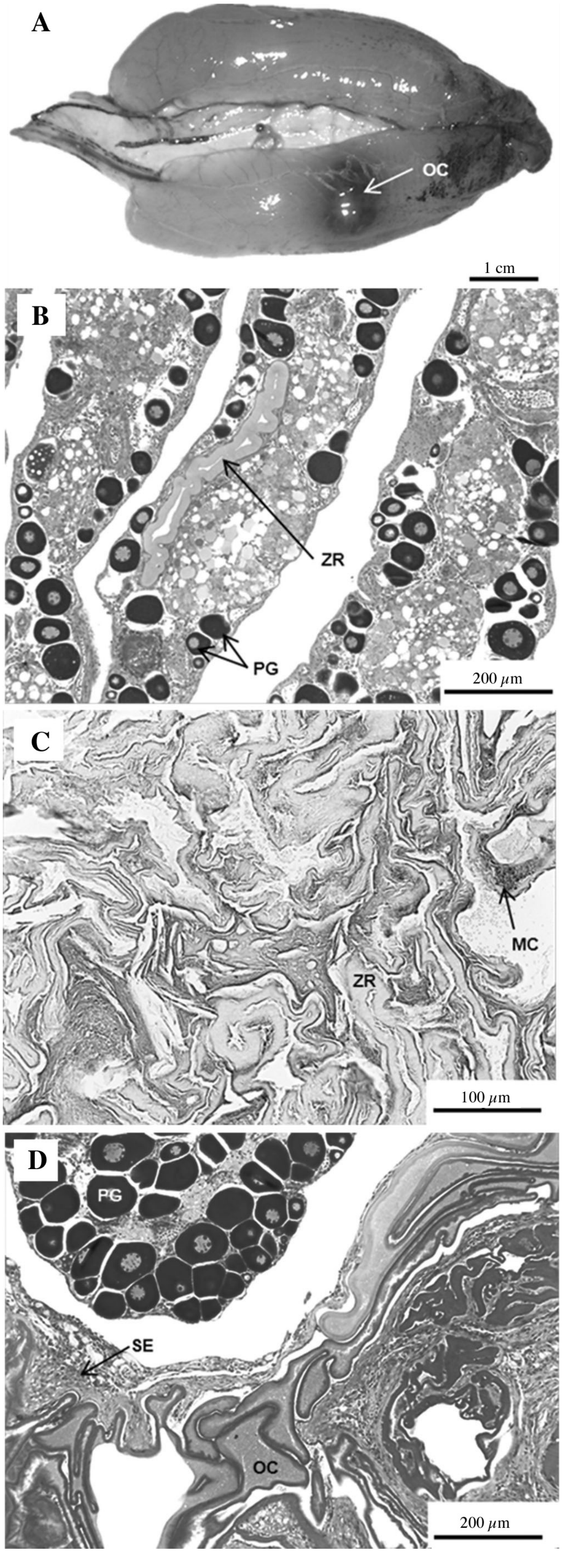

Figure 1. - Ovary of *Sarpa salpa*. **A**: Ovarian cyst observed macroscopically; **B**: Isolated cystic structure stained with toluidine blue; **C**: Aggregated cystic structure stained with haematoxylin and eosin; **D**: Detail of an aggregated cystic structure stained with toluidine blue. MC: macrophages; OC: ovarian cyst; PG: oocyte primary growth; SE: stratified epithelium; ZR: zona radiata.

females (Rideout *et al*., 2005), availability of food (Burton and Idler, 1984), environmental conditions (e.g. temperature, pH or the presence of pollutants) and population factors (e.g. changes in the proportion of sexes) (Domínguez-Petit *et al*., 2011). These cystic structures were also observed in other species along the Portuguese coast such as boarfish *Capros aper* (Linnaeus, 1758) (V. Sequeira, pers. obs.), Senegal seabream *Diplodus bellottii* (Steindachner, 1882) (C. Vendrell, pers. obs.) and forkbeard *Phycis phycis* Linnaeus, 1766 (A.R. Vieira, pers. obs.). However, the presence of a great prevalence of cysts (not sporadic), as reported in the present study, has never been reported in any species. Although the reasons that lead to this new type of degeneration are still unknown, the fact that these structures appear frequently (in all months except in October) and in high values during the months preceding the highest IG values (Tab. I), suggest that this process may be part of the reproductive strategy for *S. salpa*. Furthermore, neither environmental perturbation nor anthropogenic disturbance occurring in the sampling area could be associated to this phenomenon in the studied years. These structures showed to be relatively loose inside the ovary and were observed closely to the genital opening, being easily released after a slight pressure on the ovary. This suggests the hypothesis that the cysts can be released before spawning begins, allowing for wider space in the ovary for future feasible oocytes. Further studies are necessary to clarify this issue and determine how long the cysts remain in the ovaries.

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